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OBJECTIVE

The MHC region harbors the strongest loci for latent autoimmune diabetes in adults (LADA); however, the strength of association is likely attenuated compared with that for childhood-onset type 1 diabetes. In this study, we recapitulate independent effects in the MHC class I region in a population with type 1 diabetes and then determine whether such conditioning in LADA yields potential genetic discriminators between the two subtypes within this region.

RESEARCH DESIGN AND METHODS

Chromosome 6 was imputed using SNP2HLA, with conditional analysis performed in type 1 diabetes case subjects (n = 1,985) and control subjects (n = 2,219). The same approach was applied to a LADA cohort (n = 1,428) using population-based control subjects (n = 2,850) and in a separate replication cohort (656 type 1 diabetes case, 823 LADA case, and 3,218 control subjects).

RESULTS

The strongest associations in the MHC class II region (rs3957146, β [SE] = 1.44 [0.05]), as well as the independent effect of MHC class I genes, on type 1 diabetes risk, particularly *HLA-B*39* (β [SE] = 1.36 [0.17]), were confirmed. The conditional analysis in LADA versus control subjects showed significant association in the MHC class II region (rs3957146, β [SE] = 1.14 [0.06]); however, we did not observe significant independent effects of MHC class I alleles in LADA.

CONCLUSIONS

In LADA, the independent effects of MHC class I observed in type 1 diabetes were not observed after conditioning on the leading MHC class II associations, suggesting that the MHC class I association may be a genetic discriminator between LADA and childhood-onset type 1 diabetes.

Latent autoimmune diabetes in adults (LADA) is typically defined as initial insulin independency for at least 6 months after diagnosis and the presence of diabetes-associated autoantibodies (1). Despite such features, autoantibody screening is not carried out in routine clinical practice, resulting in frequent misdiagnosis. For instance, in a cohort of apparent type 2 diabetes cases, as many as 8–10% can actually represent misdiagnosed autoimmune diabetes cases (2,3). Hence, there is a need to identify biomarkers to aid in accurately diagnosing LADA as well as other diabetes subtypes (4).

A comprehensive analysis of the genetic etiology of LADA has, until recently, not been performed (5). Previous genetic studies have suggested that the condition

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PATHOPHYSIOLOGY/COMPLICATIONS

comprised both type 1 diabetes and type 2 diabetes components either because it is an intermediate form of diabetes or because it is a mixture of type 2 diabetes in a cohort of predominantly type 1 diabetes owing to a high false positive detection rate with use of autoantibodies when screening. There is some debate as to whether LADA is in fact a distinct clinical entity or simply a category imposed on continuous features such as age of onset and time to insulin. However, since LADA is currently defined as a slowly progressive form of type 1 diabetes (6), it is crucial to define genetic differences between childhood-onset type 1 diabetes and LADA if we are to clarify the clinical utility of identifying adult-onset autoimmune diabetes.

Previous genetic studies in LADA have shown a strong association signal in the MHC, although with diminished effect sizes compared with observations in childhood-onset type 1 diabetes (5,7). The MHC region is located on chromosome 6 and harbors >400 genes, with two main classes, MHC class I and MHC class II, which together harbor classic HLA genes (*HLA-A*, *HLA-B*, *HLA-C*, and *HLA-DRB*, *HLA-DQA*, *HLA-DQB*, *HLA-DPA*, and *HLA-DPB*, respectively). The HLA encodes cell surface proteins for antigen presentation and accounts for ~50% of the genetic heritability of type 1 diabetes, with susceptibility principally harbored within the MHC class II genes *HLA-DQB1* and *HLA-DRB1*. However, in addition to class II genes, MHC Class I genes in susceptibility to type 1 diabetes have also been suggested in previous studies (8–10); in particular, variation within the MHC class I genes *HLA-A* and *HLA-B* has been shown through conditional analysis to further increase type 1 diabetes risk (11). MHC class I markers have also been shown to be associated with younger age at diagnosis in type 1 diabetes, and given the adult-onset phenotype of LADA, we hypothesized that this genetic variation will be less enriched in LADA.

First, we aimed to recapitulate the independent effects of MHC class I variants using the SNP2HLA imputation tool followed by stepwise forward logistic regression in the same type 1 diabetes cohort that participated in a previous study (11). In addition, we set out to identify distinguishing features within the MHC between childhood-onset type 1 diabetes and adult-onset LADA by performing the same conditional analysis followed by a replication attempt in a second case/control set. Finally, we compared β regression coefficients for each disease to determine whether effect sizes differ among LADA case and control subjects versus type 1 diabetes case and control subjects.

RESEARCH DESIGN AND METHODS

Study Populations

LADA Case Subjects

A total of 1,492 LADA cases were derived from multiple cohorts across the U.K., Germany, and the U.S. Details on the participants can be found in Supplementary Table 1. All participants were diagnosed with LADA if they fulfilled the following criteria: age at diagnosis 30–70 years, testing positive for at least one diabetes-associated autoantibody (most case subjects were positive for GAD autoantibodies), and not on insulin treatment for at least 6 months after diagnosis.

Control Subjects

The LADA population-based control subjects comprised of two cohorts ($n = 2,979$). The first cohort consisted of 1,296 children and adolescents of European ancestry who did not have diabetes, aged 5–20 years, and who were enrolled in the Bone Mineral Density in Childhood Study (BMDCS) (12). The second control cohort consisted of 1,683 adults of European ancestry from a non-Hodgkin lymphoma genome-wide association study (GWAS), available in dbGaP (www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000818.v2.p1) (13). Details on the control cohorts can be found in Supplementary Table 1.

Recapitulating a Previous Study

We also leveraged 3,000 healthy adult British Birth Cohort control subjects, 2,000 individuals with childhood-onset type 1 diabetes, and 1,999 individuals with type 2 diabetes from the Wellcome Trust Case Control Consortium (WTCCC) (14) to recapitulate observations in a previous study (11). Individual data from the WTCCC are available through the consortium's Data Access Committee (http://www.wtccc.org.uk). More details on cohort information can be found in Supplementary Table 1.

Replication

A cohort of individuals from the All New Diabetics In Scania (ANDIS) and Scania Diabetes Registry (SDR) studies was used for further recapitulation and replication, including type 1 diabetes case subjects ($N = 656$), LADA case subjects ($n = 823$), and population-based control subjects ($N = 3,218$). Details on the participants can be found in Supplementary Table 1. See flowchart for overview of data sets and workflow (Supplementary Fig. 1).

Genotyping

All samples, except the WTCCC data, were genotyped using the Illumina OmniExpress genotyping chip. WTCCC type 1 diabetes and type 2 diabetes case subjects were genotyped using Affymetrix 500K, and WTCCC control subjects were genotyped

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on the Illumina 1.2M BeadChip. Quality control was performed using PLINK (15). Individuals with ambiguous sex, genotype missingness >5%, genome-wide heterozygosity (3 SDs from the mean), duplicates, and related individuals were excluded (see Supplementary Table 1 for details). Principal component analysis was performed using PLINK, and outliers were removed to exclude individuals with non-European ancestry. Single nucleotide polymorphisms (SNPs) with missing rate <5%, minor allele frequency (MAF) <1%, and Hardy-Weinberg equilibrium exact test P value $<1 \times 10^{-5}$ were removed before HLA imputation.

HLA Imputation

Starting from the genotyped SNPs, we imputed chromosome 6 using the HLA imputation software SNP2HLA along with the Type 1 Diabetes Genetics Consortium (T1DGC) reference panel (16). A marker window size of 1,000 base pairs and a posterior probability (gprob) threshold of 0.5 were used. The HLA alleles of LADA case subjects (n = 1,428) and WTCCC type 1 diabetes case subjects (n = 1,985) were imputed to both two-digit resolution and four-digit resolution for increased coverage and resolution of HLA alleles. In total, there were 5,698 SNPs, 424 HLA alleles, and 1,276 HLA amino acids. In this study, we focused on a subset of SNPs and HLA alleles that had an MAF >1% in all three control cohorts (159 HLA alleles and 5,506 SNPs remained).

Power Calculations

Power calculations were performed using the Genetic Association Study (GAS) Power Calculator (http://csg.sph.umich.edu/abecasis/cats/). Assumptions included a multiplicative model, a disease incidence of 0.0036, 1,428 case and 2,979 control subjects, and a significance level of 8.83×10^{-6}, based on a Bonferroni correction for the 5,665 variants tested (Supplementary Table 2).

Recapitulation of a Previously Published Conditional Analysis for Type 1 Diabetes

Logistic regression using SNPTEST (17) was used to test all HLA alleles and SNPs with MAF >1% in all three control cohorts. Sex and the 12 broad geographical regions, provided by the WTCCC, were included as covariates in the analysis. The analyses were performed in the WTCCC type 1 diabetes versus control data sets using forward stepwise conditional logistic regression until there were no significant signals remaining after correction for multiple testing.

Conditional Analysis in Subjects With LADA Versus Population-Based Control Subjects

Conditional logistic regression was performed using SNPTEST in the LADA versus population-based control subjects, including sex and the first four principal components as covariates.

Replication

To further validate MHC class I independent effects in type 1 diabetes and lack of MHC class I independent effects in LADA, we implemented approximate conditional and joint (COJO) analysis in GCTA (18) on summary statistics from the Swedish replication cohort. Association analysis was performed using SNPTEST, and sex and the first four principal components were used as covariates. There were 656 case subjects with type 1 diabetes vs. 3,218 population-based control subjects and 823 case subjects with LADA vs. 3,211 population-based control subjects.

Sensitivity Analysis

We performed sensitivity analysis to determine whether the lack of independent type 1 diabetes–associated signals in MHC class I genes in LADA case subjects could be due to a lack of power. We randomly sampled 1,428 type 1 diabetes case subjects and 714 type 1 diabetes cases subjects (subsets equating to the same size as the LADA cohort and half the size of the LADA cohort, respectively) and 2,219 control subjects to determine whether the type 1 diabetes–associated signals could be still be detected. Stepwise conditional logistic regression using SNPTEST was performed as described above. To test the hypothesis that LADA is a mixture of type 1 diabetes and type 2 diabetes cases, we performed a conditional analysis in 714 randomly sampled type 1 diabetes cases and 714 randomly sampled type 2 diabetes cases (total n = 1,428 cases) and 2,219 WTCCC control subjects.

Further Validating Independent Signals

PLINK was used to calculate pairwise linkage disequilibrium (LD) between variants to further validate that the associated variants were truly independent of each other. For confirmation of the independent association of *HLA-B*39*, the specific *HLA-B*39* subtype *HLA-B*3906* was tested in the WTCCC type 1 diabetes case subject (n = 1,985) versus control subject (n = 2,219) data set with *DQB1*0402* and *DQB1*0501* as covariates.

Ethics Approval

This study was approved by local institutional ethics review boards.

RESULTS

Confirming Independent Effects of MHC Class I Signals in WTCCC Subjects With Type 1 Diabetes Versus Control Subjects

Before conditioning, we observed rs3957146 as the strongest association signal in analysis of subjects with type 1 diabetes versus WTCCC control subjects ($P = 8.94 \times 10^{-165}$) (Fig. 1*A*). rs3957146 is in strong LD with a classical HLA subtype allele, *HLA-DQB1*0302* (r^2 = 0.99). After conditioning on the top signal, rs3957146, and subsequent independent MHC class II signals (*HLA-DQB1*0201* and rs9268633), we observed the reported independent significant association of MHC class I variants rs1610649 (*HLA-G*, $P = 6.89 \times 10^{-23}$) and *HLA-B*39* ($P = 6.89 \times 10^{-23}$) (Fig. 1*B*, Table 1, Supplementary Table 3, and Supplementary Fig. 2). Conditioning on these variants in addition to the MHC class II variants also demonstrated significant association with the *HLA-A* locus (rs9259852, $P = 2.04 \times 10^{-8}$).

Conditional Analysis in Subjects With LADA Versus Population-Based Control Subjects

We then went on to perform stepwise conditional analysis in 1,428 LADA case and 2,979 control subjects. Similar to observations in the type 1 diabetes versus WTCCC control data set, before conditioning on any variants, the strongest association signal in LADA case subjects versus population-based control subjects was also rs3957146 ($P = 1.80 \times 10^{-68}$) (Fig. 2*A*). Although we had 98% power to detect *HLA-B*39* with an allele frequency of 2% and an odds ratio of 2.5 (Supplementary Table 2), when conditioning on the most highly significant MHC class II alleles (rs3957146, *HLA-DRB1*03*, rs9269081, *DRB1*0404*, and

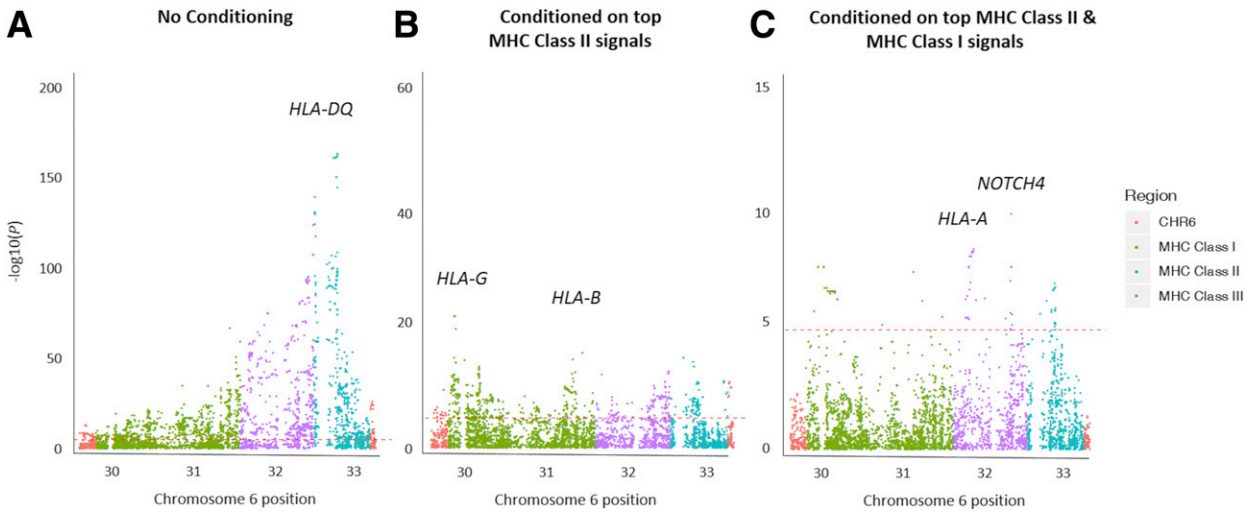

Figure 1—Conditional analysis in 1,985 type 1 diabetes case and 2,219 WTCCC control subjects. *A*: Logistic regression analysis without conditioning on MHC class II alleles. *B*: Logistic regression analysis conditioning on MHC class II alleles. *C*: Logistic regression analysis conditioning on MHC class II and MHC class I signals.

*DQB1*0602*), there were no remaining independent signals in the MHC class I region reaching significance after correction for multiple comparisons ($P < 8.83 \times 10^{-6}$) (Fig. 2*B*). Furthermore, we also noted independent effects in the MHC class III region (rs2143462, $P = 8.24 \times 10^{-8}$) and the MHC class II region (*HLA-DPA1*02*, $P = 1.62 \times 10^{-6}$, and *HLA-DPB1* variant rs3130192, $P = 5.32 \times 10^{-6}$), which are known to be associated with type 1 diabetes (19). Here, *HLA-DPB1* variant is in strong LD with rs2301225 ($r^2 = 0.85$) and is independently associated with type 1 diabetes. MHC class I variants were not observed to be independently associated with LADA after correction for multiple comparisons (Table 1, Supplementary Fig. 2, and Supplementary Table 4).

Sensitivity Analysis in Reduced Sample of Subjects With Type 1 Diabetes Versus Control Subjects

To ensure that the lack of significant associations with MHC class I genes in the LADA cohort was not explained by reduced power, we conducted a sensitivity analysis by systematically decreasing the sample size of the type 1 diabetes versus WTCCC control cohort to match the size

Table 1—Comparison of β-coefficients between conditional analyses in type 1 diabetes and LADA cohorts

				WTCCC type 1 diabetes case vs. WTCCC control subjects				LADA case vs. control subjects				
SNP/HLA allele	Locus	Position	Alleles (risk/other)	RAF, case subjects	RAF, control subjects	β	SE	RAF, case subjects	RAF, control subjects	β	SE	*P*
rs3957146	*HLA-DQA2*	32789508	T/C	0.385	0.113	1.44	0.05	0.251	0.101	1.14	0.06	1.22×10^{-4}
DQB1*0201	*HLA-DQB1*	32739039	P/A	0.338	0.140	1.56	0.06	0.209	0.119	1.05	0.07	3.17×10^{-8}
rs9268633	*HLA-DRA*	32514451	G/A	0.983	0.803	1.46	0.10	0.919	0.812	0.83	0.06	6.58×10^{-8}
rs1610649	*HLA-G*	29876896	G/A	0.616	0.582	0.61	0.06	0.592	0.586	0.16	0.05	8.33×10^{-9}
B*39	*HLA-B*	31431272	P/A	0.043	0.016	1.36	0.17	0.023	0.019	0.58	0.19	2.22×10^{-3}
DRB1*0404	*HLA-DRB1*	32660042	P/A	0.082	0.048	1.04	0.13	0.037	0.035	1.01	0.14	0.88
rs17427599	*HLA-DQB1*	32775342	T/C	0.849	0.755	0.59	0.09	0.818	0.775	0.31	0.07	1.41×10^{-2}
rs2301225	*HLA-DPA1*	33143838	T/C	0.941	0.891	0.72	0.11	0.924	0.886	0.42	0.08	2.74×10^{-2}
rs397081	*NOTCH4*	32300595	T/C	0.095	0.045	0.79	0.12	0.075	0.054	0.64	0.10	0.34
rs9262545	*MUC22*	31101041	A/G	0.913	0.881	0.67	0.11	0.862	0.858	0.09	0.07	8.65×10^{-6}
rs9262547	*MUC22*	31101206	T/A	0.135	0.119	1.59	0.19	0.137	0.142	−0.09	0.07	1.07×10^{-16}
rs9259852	*HLA-A*	30004400	C/T	0.977	0.959	1.00	0.18	0.968	0.963	0.19	0.13	2.64×10^{-4}
rs9269081	*HLA-DRA*	32549078	A/C	0.890	0.735	0.57	0.11	0.821	0.685	0.71	0.05	0.25
rs1978029	*HLA-DQB2*	32839688	C/T	0.651	0.537	0.36	0.08	0.565	0.475	0.39	0.05	0.75

β-Coefficients and SEs are calculated for the risk allele frequency (RAF) from stepwise regression conditional on all SNP/HLA alleles (first column) in 1,985 type 1 diabetes case vs. 2,219 control subjects. β-Coefficients and SEs for LADA case vs. control subjects correspond with maximum effect size in conditional analysis. *P* values are derived from two-sample *Z* test to formally test whether β-coefficients are significantly different. Forest plot is shown in Supplementary Fig. 2. Three independent signals appeared in both type 1 diabetes and LADA conditional analyses (rs3957146, *HLA-DRB1*0404*, and rs9269081); however, of these three signals, only rs3957146 had a significant difference in effect size between type 1 diabetes and LADA (interaction *P* value = 4.15×10^{-10}).

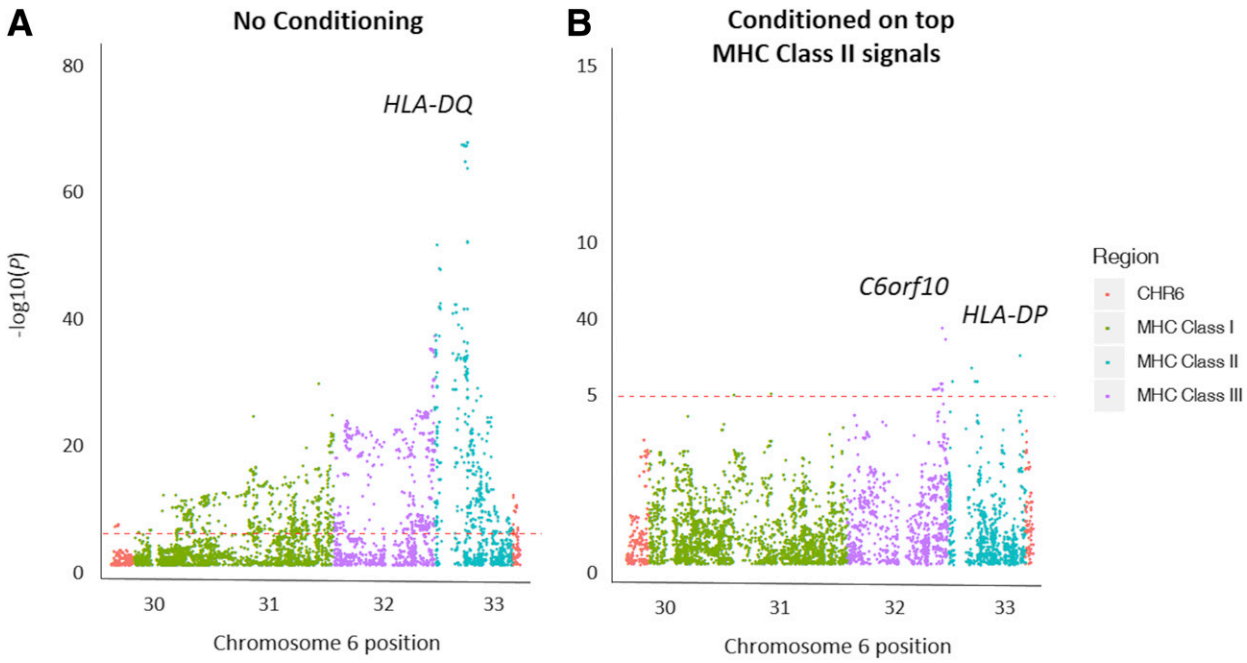

Figure 2—Conditional analysis in 1,428 LADA case and 2,979 WTCCC control subjects. *A*: Logistic regression analysis without conditioning on MHC class II alleles. *B*: Logistic regression analysis conditioning on MHC class II alleles.

of the discovery LADA cohort ($n = 1{,}428$ type 1 diabetes case and 2,219 control subjects) and performing conditional analysis. Independent significant association signals at *HLA-G* ($P = 1.37 \times 10^{-17}$), *HLA-B* ($P = 5.58 \times 10^{-14}$), and *MUC22* (rs9262545, $P = 3.36 \times 10^{-9}$; rs9262547, $P = 9.69 \times 10^{-14}$) were still observed in this reduced type 1 diabetes sample size (Supplementary Table 5), although these signals were missing in the comparatively sized LADA versus control subjects data set. Similarly, independent significant association signals at *HLA-B* ($P = 1.26 \times 10^{-10}$), *HLA-G* ($P = 0.002$), and *MUC22* (rs9262545, $P = 1.57 \times 10^{-5}$) remained after further reduction of the type 1 diabetes cohort size to equate with half the LADA cohort size (Supplementary Table 6).

Sensitivity Analysis in a Randomly Mixed Cohort of Type 1 Diabetes and Type 2 Diabetes Case Subjects Versus Control Subjects

Another explanation for the lack of independent, significant associations across MHC class I genes in LADA could be that the LADA cohort simply represents an ~50/50 mixture of misdiagnosed type 1 diabetes and type 2 diabetes cases. Therefore, we randomly sampled 714 type 1 diabetes case subjects, 714 type 2 diabetes case subjects, and 2,219 control subjects, creating a "mixture" cohort. We performed the same conditional analysis described above and observed that the *HLA-B*, *HLA-G*, and two *MUCC2* signals in the MHC class I regions remained independently significant in this mixed cohort, driven by the type 1 diabetes case subset (Supplementary Table 7).

Replication

We leveraged summary statistics data from Swedish cohorts to attempt replication of our findings. In type 1 diabetes case versus control subjects, the strongest association was rs9275206 ($P = 6.35 \times 10^{-89}$), which is in strong LD with *HLA-DQB1*0302* ($r^2 = 0.99$). After conditioning on rs9275206 and subsequent top signals (Supplementary Table 8), we again observed significant association signals at the *HLA-G* ($P = 1.74 \times 10^{-10}$) and *HLA-B* (1.10×10^{-9}) loci. However, when conditional analysis was performed in LADA case versus control subjects, there were no such signals across MHC class I genes and very sparse signals in the MHC class II region (Supplementary Table 9). Furthermore, we observed a significant association signal at the *NOTCH4* (rs397081, $P = 1.11 \times 10^{-10}$) locus, the *MUC22* locus (rs9262545, $P = 7.83 \times 10^{-11}$, and rs9262547, $P = 7.17 \times 10^{-17}$), and the *HLA-A* locus (rs9259852, $P = 5.84 \times 10^{-14}$) (Fig. 1*C*). Notably, rs9259852 is in strong LD with the classic HLA subtype allele, HLA-A*32 ($r^2 = 0.96$).

Further Validating HLA*B*39

It has been shown that the *HLA-B*3906* allele is associated with a high risk of diabetes only for specific HLA-DR/DQ haplotypes, *DRB1*0801-DQB1*0402* and *DRB1*0101-DQB1*0501* (20). With specific conditioning on these HLA-DR/DQ haplotypes in the WTCCC type 1 diabetes case and control cohort, the independent significant association of the more specific *HLA-B*3906* subtype still remained (odds ratio 4.57 [95% CI 3.08–6.80]; $P = 5.84 \times 10^{-14}$).

CONCLUSIONS

The main objective of this study was to perform conditional analysis of the HLA region in LADA, which has been underexplored to date in this disease context. The few genetic studies in LADA (5,21,22) only focused on the HLA class II *DRB1* and *DQB1* haplotypes. Such studies, in populations of both European and Chinese ancestry, show that type 1 diabetes risk haplotypes are less frequent in LADA compared with childhood-onset type 1 diabetes case subjects, whereas type 1

diabetes protective haplotypes are more frequent in LADA, suggesting that LADA is a genetically attenuated form of type 1 diabetes. By extending the analysis of HLA in LADA beyond the MHC class II region, we were able to observe further genetic differences between LADA and childhood-onset type 1 diabetes.

Although previous studies have reported MHC class I independent effects in type 1 diabetes, those studies directly used HLA-typed case and control subjects. Given the cost and challenges of direct HLA typing, we utilized the imputation tool SNP2HLA on genotyping data. SNP2HLA has been commonly used in the field to assess the genetics of autoimmune diseases (16,23–25). Furthermore, given that this approach differs from that of Nejentsev et al. (11), it was crucial to first ensure that we could recapitulate the previously reported type 1 diabetes observations in the same cohort. First, we leveraged the WTCCC type 1 diabetes case and control data set, as a positive control, with previous studies identifying MHC class I independent type 1 diabetes associations in the MHC class I region (8,10,11,26). Since these studies were reported, imputation tools have allowed the analysis of the HLA region more cheaply and, in general, more practically. Before investigating MHC class II independent LADA associations in the MHC class I region, given the difference in our analytical approach, we recapitulated the observations in previous studies (10,11) by leveraging the same WTCCC type 1 diabetes and control data sets. We confirmed that MHC class I variants are significantly associated with type 1 diabetes, independent of the MHC class II region using this imputation-based approach followed by stepwise conditional logistic regression. The conditional analysis was repeated in the LADA cohort, which consisted of case and population-based control subjects. Crucially, there were no significant independent effects in the MHC class I region remaining after correction for multiple comparisons; furthermore, this observation was replicated in a separate Swedish cohort of type 1 diabetes case, LADA case, and population-based control subjects.

The MHC class I variant *HLA-B*39* is an established locus associated with type 1 diabetes risk (10,11,27). More specifically, studies suggested a strong association with type 1 diabetes for the subtype *HLA-B*3906,* which is now used in type 1 diabetes genetic risk scores to predict type 1 diabetes diagnosis (23). It has also been shown that the *B*3906* allele significantly enhances the risk of type 1 diabetes when present on specific *HLA-DR/DQ* haplotypes (e.g., *DRB1 0801-DQB1 0402* and *DRB1 0101-DQB1 0501*). The frequency of *HLA-B*3906* is different among different populations and here did not survive our filter of having an MAF $>$1% in the replication control cohort of Swedes. Thus, it was excluded in the analyses across the three cohorts. However, we confirmed that the *HLA-B *3906* allele remained significantly associated with type 1 diabetes after conditioning on the presence of the *DRB1 0801-DQB1 0402* and *DRB1 0101-DQB1 0501* haplotypes. Additionally, *HLA-B*3906* is associated with younger age-at-diagnosis in type 1 diabetes (9,11). A recent study using a NOD mouse model showed that *HLA-B*3906* mediates the development of $CD8^+$ T cells required for type 1 diabetes onset; moreover, in the context of reduced immunological tolerance to insulin, *HLA-B*3906*-transgenic NOD mice develop type 1 diabetes at an accelerated rate (28). The lack of an independent *HLA-B*39* association observed in the adult-onset phenotype of LADA further confirms the link between *HLA-B*39* with autoimmune progression with earlier onset of clinical disease.

HLA-B associations have been confirmed in a previous study (26), as well as associations around *HLA-G*, which is expressed in human pancreas (29) and may play a role in autoimmune progression (30). However, the MHC class I variant rs1619379, located in *HLA-G* and ~100 kb telomeric of *HLA-A*, may be less informative compared with *HLA-A* variants in predicting type 1 diabetes risk (10). This particular MHC class I variant was independently significant in the down-sampled type 1 diabetes cohort, but is in strong LD with *HLA-G* variants, rs1610649 and rs2735028, which were significantly associated in the full type 1 diabetes set, the mixture cohort consisting of type 1 diabetes and type 2 diabetes case subjects, and the type 1 diabetes Swedish replication cohort. Additionally, the MHC class I variants located in the *MUC22* locus have not been replicated in separate cohorts and likely form haplotypes with HLA class I alleles.

One limitation of this study was that we only tested variants with an MAF $>$1% in all three control cohorts, which resulted in filtering out many informative alleles such as *HLA-B*3906*. By filtering to include only common alleles, we limited potential discrepancies between populations and were able to replicate our observations across cohorts with different frequencies of known risk variants. Furthermore, our study is limited in power to assess the underlying continuous traits of age at onset, time to insulin, and autoantibody titer; future well-designed, large studies are needed to enable those analyses. With larger and more complete data in individuals, we can then formally test the many competing hypotheses regarding the state of LADA in the field. The hypothesis that LADA exists as a disease different from type 1 diabetes with both overlapping genes and distinct genes is unlikely, as we did not clearly observe distinct susceptible loci that were unique to LADA in this study or our previous GWAS (5). Future studies leveraging case subjects diagnosed with type 1 diabetes and LADA across the age of onset range will be crucial to test the remaining hypotheses, which are: *1*) LADA is type 1 diabetes with misdiagnosed type 2 case subjects who are false positive for autoantibodies, *2*) LADA cases are essentially type 1 diabetes at later onset with lower rates of progression, *3*) LADA is a form of diabetes where case subjects have both type 1 and type 2 risk alleles present at the individual level. This first hypothesis (LADA is type 1 diabetes with misdiagnosed type 2 case subjects who are false positive for autoantibodies) motivated the sensitivity analysis, in which we randomly sampled case subjects from the WTCCC type 1 diabetes and type 2 diabetes cohort to create a random LADA cohort under the assumption that the LADA group would be a "mixture" of actual type 1 diabetes and type 2 diabetes case subjects. In this analysis, we still observed the same independent effects of MHC class I variants, showing that the type 1 diabetes signature remained in the "mixture" cohort despite not being observed in LADA. However, we recognize that type 1 diabetes case subjects were sampled from the cohort of childhood-onset type 1 diabetes and that properly testing this hypothesis would require that LADA cases be ascertained from another cohort but also further stratified by autoantibody titer.

Although the underlying populations from which the type 1 diabetes and LADA sets were derived are the same, we addressed whether the LD structure in the HLA region could be different between the two sample sets, which in turn could have resulted in inaccurate imputation. However, when we calculated LD for the MHC region in these data sets, we found it to be highly correlated (Pearson correlation coefficient = 0.97). Future studies are needed to address this question in-depth as well as validate these findings in a cohort directly typed for MHC class II and MHC class I HLA alleles. Additionally, for further delineation of this putative distinguishing genetic feature between LADA and childhood-onset type 1 diabetes, it will be crucial to investigate how the HLA profile compares across the diabetes age continuum stratified for different autoantibody positivity status. A previous study observed different independent effects of MHC class I variants to GAD autoantibodies and insulinoma-associated antigen-2 autoantibodies (31). Additionally, studies have shown that children with type 1 diabetes who are positive for a single autoantibody are more like to show type 2 diabetes features (32,33), for instance, a significant association with type 2 diabetes GWAS-implicated variants. Overall, our results point to key differences in the genetic signature in the MHC region, especially class I markers, between LADA and childhood-onset type 1 diabetes. This study highlights the clinical utility of genetic screening in adult-onset diabetes that may be autoimmune in origin. The potential of defining these subjects who are at risk for rapid loss of insulin secretion using genetic characteristics could enable targeted immune-based, disease-modifying therapy.

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